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Document Contact	Kelly Sartor
Area	Laboratory-Blood Bank
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Testing of Eluates by the Tube Method - Dearborn Blood Bank

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

This document is to provide instructions to test an eluate by the tube method.

II. PRINCIPLE:

Once an eluate has been prepared as described in Transfusion Medicine policy, Preparation of Eluate, it will be tested for antibody activity against a panel of RBCs. Some eluates will demonstrate identifiable antibody activity, some will demonstrate no activity, and others will demonstrate non-specific reactivity. Non-specific reactions are frequently observed in the eluates of patients with warm autoantibodies. Those eluates that demonstrate identifiable antibody activity are most often encountered in hemolytic transfusion reactions and in cases of hemolytic disease of the newborn (HDN).

III. SCOPE:

A. The gel method is the standard method for testing eluates at this facility. Refer to Transfusion Medicine policy, Testing of Eluates by the Gel Method.

B. Eluates should be tested by the tube method as described in this document only if:

1. The eluate was tested by the gel method, and
2. Non-specific reactions were observed in the gel eluate, and
3. The patient does not have a warm autoantibody.

IV. DEFINITIONS/ACRONYM:

A. **Standard panel**: a commercially prepared panel that usually consists of 11 vials of human RBCs. It is usually performed on patients who do not have a historical antibody record.

B. **Selected cell panel**: a panel that is pre-selected based on the antigenic profile of the test RBCs.

C. **RPM**: revolutions per minute.

V. POLICIES:

A. Alternate Method

1. The tube method is the alternate method of testing eluates and shall only be performed as indicated in the *Scope* section of this document.

B. Appropriate Test Cells

1. Generally, eluates tested by the tube method should be tested against a standard, commercially prepared panel of 2 - 4% test cells.
2. The eluate may be tested against a selected cell panel of 2 - 4% test cells in the following cases:
    a. To test for the presence of additional antibodies, when known antibody specificities have been previously identified.
    b. When a smaller than normal volume of eluate is prepared; e.g., neonatal samples. In this case it may be necessary to test the eluate against a smaller number of panel cells, or against a set of screening cells.
    c. If a patient develops a positive Direct Antiglobulin Test (DAT) after the transfusion of ABO plasma-incompatible components, then the eluate should be tested against 2 examples each of A_1, B, and O test RBCs.

VI. SPECIMEN COLLECTION AND HANDLING:

A. The preferred sample is a 6 ml EDTA sample or a cord blood specimen with affixed identifying label. The eluate should be prepared while the sample is still fresh. If a delay in preparing the eluate is necessary, the specimen should be stored at 1-10° C, preferably for not longer than 72 hours. A volume of 1 mL washed RBCs is generally required, but smaller volumes may be sufficient; see Transfusion Medicine Policy, Preparation of an Eluate.

B. The prepared eluate may be tested up to seven (7) days after preparation, provided that it is stored at 1-10° C and that turbidity has not developed. Refer to the Gamma Elu-Kit II manufacturer's insert for further information.

VII. REAGENTS:

A. 2% - 4% test cells; refer to the policy Appropriate Test Cells.

B. Coombs control cells (IgG coated cells)

C. Antihuman globulin (AHG) reagent, monospecific Anti-IgG

D. Working Wash Solution: Prepared from concentrated wash Solution by diluting 1 in 10 with laboratory reagent-grade water, as described in Transfusion Medicine policy, Preparation of Eluate. Once diluted, the working wash solution may be stored at 1°C to 10°C for as long as it shows no obvious signs of turbidity and is not causing hemolysis of RBCs.

VIII. EQUIPMENT:

A. table top centrifuge

B. lighted viewing mirror

C. heat block incubator (37°C)

D. calibrated timer

E. automatic cell washer

IX. SUPPLIES:

A. 12 x 75 mm test tubes

B. disposable pipettes

C. gauze

X. QUALITY CONTROL (QC):

A. Antibody Screen of the Last Wash Supernate

1. An antibody screen of the last wash supernate, obtained during the eluate's preparation must be tested in parallel with the eluate.
2. The purpose of this antibody screen is to assure that antibody in the eluate has been derived from a bound state on the RBCs, and is not merely the result of inadequate washing of the RBCs during the eluate's preparation.
3. If the eluate will be tested against group A and B cells, then the last wash must also be tested against group A and B cells (a set of reverse cells).
4. Directions to perform this QC are included in the procedure section.
5. Passing QC: The Quality Control- Antibody Screening Test should be non-reactive. If the last wash was tested against a and b cells (a set of reverse cells), then this test should also be non-reactive.
6. Failing QC: If the Quality Control- Antibody Screening Test is reactive (or if the last wash was tested against a and b cells and is reactive), then the eluate generally cannot be interpreted.
    a. A fresh eluate should be prepared and washing the RBCs additional times; refer to Transfusion Medicine policy, Preparation of the Eluate.
        i. If the quality control is still reactive, even after preparing a fresh eluate with additional washing, this may indicate that residual serum antibody was present. The eluate may then be considered contaminated, and

interpretation may not be valid.

ii. Such reactivity may also occur if the antibody coating the RBCs has low affinity for its corresponding antigen and eluates during the washing process. This may be minimized by washing in 1°C to 10°C Working Wash Solution, although in most cases satisfactory eluates can be made washing at room temperature.

7. Check Cells: IgG coated cells (Coombs Control cells) must be added to all AHG phase results that are negative. If a test result with IgG coated cells is negative, then the test must be repeated.

XI. PROCEDURE:

A. Proceed from procedure, Preparation of Eluate.
Note: Verify that supernatant is clear, free of supernatant and cellular debris. If necessary re-centrifuge supernatant additional 5 minutes and transfer the supernatant to a new 12 X 75 mm test tube labeled with the patient's name.

B. Label 12 x 75mm tubes with the following information:
   1. The patient's last name.
   2. Label each tube with the identification of the cells against which the eluate will be tested.
   3. Label additional wells for the last wash quality control (3% Ortho Surgiscreen and/or reverse cells).

C. Place 1 drop of panel cells, 3% Ortho Surgiscreen, or reverse cells (if indicated) in the correspondingly labeled tubes.

D. Wash the test cells with 5-10 drops of normal saline. Centrifuge for 30 seconds at 3400 RPM, decant the saline, and blot the tubes dry.

E. Add 2 drops of the eluate to the dry cell button in each correspondingly labeled tube. **Do not add LISS**.

F. Add 2 drops of the last wash supernate (saved during the preparation of the eluate) to the dry cell button in each correspondingly labeled tube (3% Ortho Surgiscreen and/or reverse cells).

G. Mix the tubes thoroughly and incubate at 37°C ± 1°C for 15 minutes.
Note: Incubation may be extended up to 30 minutes, and may enhance reactivity.

H. After incubation, wash all tubes with the Working Wash Solution as follows:
   1. Add 5-10 drops Working Wash Solution to the tubes and mix.
   2. Centrifuge for 30 seconds at 3400 RPM.
   3. Decant the Working Wash Solution completely and blot the tubes dry.

I. Add 2 drops Anti-IgG AHG reagent to each dry cell button.

J. Mix well and centrifuge for the AHG time calibrated for the centrifuge.

K. Resuspend the cells by shaking gently. Read, grade, and record the graded reactions of the eluate and of the last wash at the AHG phase on the cell antigrams. Do not read

microscopically.

L. Add Coombs control cells to all AHG phase results that are negative. Agitate tubes to mix and centrifuge according to calibrated time.

M. Gently resuspend the cell button and read, grade, and record the Coombs control cell test results on the cell antigrams.

    1. Coombs control cells must react positive (any strength); otherwise the test must be repeated.

N. Interpret the eluate results.

XII. INTERPRETATIONS

A. Eluates can not be interpreted unless the quality control testing of the last wash is non-reactive. See Quality Control section above.

B. Eluates will be interpreted in the same manner as an antibody panel, as described in Transfusion Medicine policy, Interpretation of Antibody Investigations. Most often when a specific alloantibody is identified in the eluate, that same antibody activity is present in the serum. The reactions observed in the gel testing will be interpreted and reported in one of three ways:

    1. ENR: if the eluate is non-reactive, or
    2. ENS, if the eluate is non-specific, or
    3. Reactive, with a red cell antigen specificity. If a specificity is identified, consider the patient's transfusion and medical history, and the DAT results to determine whether the antibody is an autoantibody of apparent specificity, or an alloantibody, or a passively acquired antibody. Consult the Supervisor or Medical Director if necessary.

XIII. LIMITATIONS:

A. Even though RBCs used for the elution may have a positive DAT, in some cases no antibody activity will be detected in the eluate. This may be because the IgG coating on the red cells is not directed at RBC antigens, or that the antibody requires certain drugs to be present in the test system for detection.

B. RBCs having a positive DAT attributable to only bound complement will normally yield an eluate showing no antibody reactivity.

XIV. REFERENCES:

1. AABB, *Technical Manual*, current edition.
2. Immucor / Gamma ELU-KITTM II, Manufacturer's Insert, 09/2010.

Approval Signatures

Step Description	Approver	Date
	Jeremy Powers: Chief, Pathology	6/21/2022
Policy and Forms Steering Committe (if needed)	Gail Juleff: Project Mgr Policy [IH]	6/17/2022
Policy and Forms Steering Committe (if needed)	Kelly Sartor: Supv, Laboratory	6/14/2022
	Kimberly Geck: Dir, Lab Operations B	6/13/2022
	Kelly Sartor: Supv, Laboratory	6/9/2022
	Kelly Sartor: Supv, Laboratory	6/9/2022